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Pathogenesis and Therapy of Liver Disease in Protoporphyria

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Protoporphyria is an inherited disorder in man characterized by the overproduction of protoporphyrin, a compound that is excreted by the liver. Hepatobiliary disease may occur in protoporphyria, and several cases have been reported in which death was due to liver disease. Based on the histological evaluation of liver biopsy specimens from 18 patients, 6 of whom died with cirrhosis and liver failure, we speculate that liver disease in this condition is caused by protoporphyrin deposits in hepatobiliary structures. These deposits are composed of crystals and have a characteristic birefringence when examined by polarization microscopy.

One patient with early liver damage was given cholestyramine and vitamin E in an attempt to reduce the amount of protoporphyrin which the liver excreted daily. Liver function tests returned to normal, and red cell and plasma protoporphyrin levels decreased. A repeat liver biopsy after one year of therapy showed healing, with decrease of the protoporphyrin deposits.

Liver disease in protoporphyria may be treated by directing therapy toward the metabolic abnormality.

INTRODUCTION

Protoporphyria is an inherited disorder of porphyrin metabolism in man in which there are increased levels of protoporphyrin in red cells, plasma, and feces. Protoporphyrin is a dicarboxylic acid porphyrin that is poorly water-soluble at physiological pH. It is excreted solely into bile [1]. Urinary excretion of protoporphyrin does not occur, even in the face of biliary obstruction.

Recent studies have demonstrated that the basic defect in protoporphyria is a deficiency in the activity of heme synthetase (ferrochelatase), the enzyme which catalyzes the chelation of iron to protoporphyrin to form heme (Fig. 1). Deficient heme synthetase activity has been demonstrated in various tissues from patients with protoporphyria, including liver [2-6]. Although the developing red cell is probably the major site of porphyrin overproduction in the disease [7], studies with labeled precursors of protoporphyrin indicate that the liver may contribute a variable fraction [8,9].

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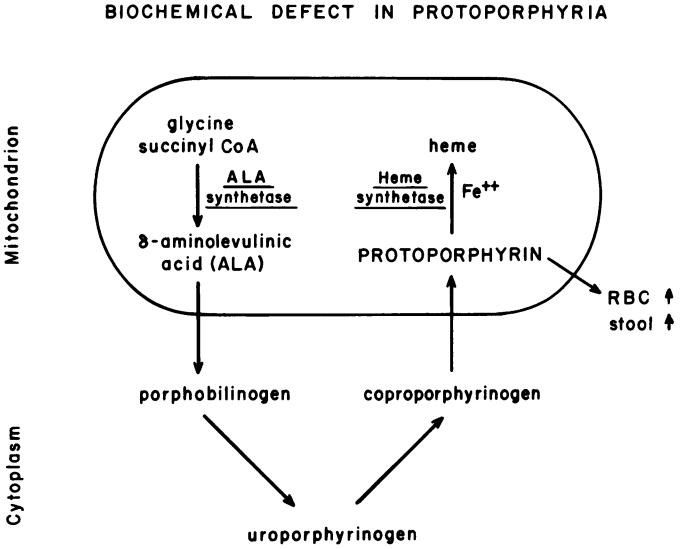

FIG. 1. Heme biosynthetic pathway. In protoporphyria, the basic defect is a deficiency of heme synthetase activity, which catalyzes chelation of iron to protoporphyrin to form heme. This leads to excessive accumulation and excretion of protoporphyrin.

Photosensitivity, with onset in early childhood, is the major clinical manifestation of the disease. It is produced when protoporphyrin absorbs light of wavelength 400–410 nm and initiates damage in skin. Hepatobiliary disease is less common but may be a critical complication, and 15 deaths from hepatic failure have been reported [10–22]. These cases are summarized in Table 1. The patients have generally been over the age of 30. Cripps and coworkers recently reported the youngest case of fatal liver disease in an 11-year-old boy with protoporphyria [21]. Porter and Lowe previously reported a 6-year-old boy with protoporphyria and cirrhosis who improved following splenectomy [23]. Thus liver disease is less common in younger patients, but certainly occurs.

The clinical histories of the patients indicated that the liver disease was not associated with alcoholism, viral hepatitis, drug use, or exposure to toxins. Biochemical studies showed direct hyperbilirubinemia (total levels 7.0 to 22.0 mg/100 ml), mild to moderate increases in the serum transaminase (60 to 475 units), and less than twofold elevations in the serum alkaline phosphatase. Hepatitis B infection, α_1-antitrypsin deficiency, iron overload, and chronic active hepatitis associated with smooth muscle antibody were excluded in those patients in whom studies were performed.

Liver biopsy specimens from the fatal cases demonstrated cirrhosis with massive deposits of dark brown pigment. When examined by polarizing microscopy, the pigment deposits were found to have a distinctive birefringence [24], and on electron microscopy, were shown to be crystalline in nature [17]. Although the chemical composition of the birefringent pigment deposits has not been completely defined, they are thought to be composed in major part of protoporphyrin, since the protoporphyrin content of the liver specimens has been very high (Table 1). Thus the current speculation is that liver disease occurs in protoporphyria because protoporphyrin deposition in hepatobiliary structures causes hepatic inflammation and fibrosis.

TABLE I
Cases of Protoporphyria Dying with Cirrhosis and Hepatic Failure

Author and Year[a]	Patient Age	Patient Sex	Protoporphyrin Levels Red cell (μg/100 ml)	Protoporphyrin Levels Plasma (μg/100 ml)	Liver (μg/g wet wt.)	Hepatic Pigment Deposits Present	Hepatic Pigment Deposits Birefringent
Barnes et al. (1968)	42	M	—	—	4900	+	NE[b]
Donaldson et al. (1971)	56	M	6000	—	208	+	NE
	58	M	—	—	—	+	NE
Schmidt and Stich (1971)	40	F	3150	—	760	+	NE
Iwanov et al. (1972)	42	M	3793	—	—	+	NE
Scott et al. (1973)	43	F	4000	690	3200	+	NE
Thompson et al. (1973)	31	F	—	—	—	+	+
	31	F	2000	—	—	+	+
Meffert et al. (1974)	44	F	3180	—	—	+	NE
Bloomer et al. (1975)	33	F	4516	790	1787	+	+
Meyer et al. (1975)	16	M	7749	—	—	+	+
Pimstone et al. (1976)	29	M	—	—	1600	+	+
Nicholson and Zawirska (1976)	55	M	4700	—	819	+	NE
Cripps et al. (1977)	11	M	8700	870	57,500	+	+
Singer et al. (1978)	60	M	3170	56	—	+	+
Normal levels			<50	<10	trace		

[a] Year of report. See references [10–22] for individual cases.
[b] NE Hepatic pigment deposits were not examined for birefringence.

These observations indicate that the liver plays a central role in protoporphyria. The following report summarizes the experience in the Yale Liver Study Unit with hepatic aspects of the disease.

RESULTS

Histological Features of Liver Biopsy Specimens in Protoporphyria

Liver biopsy specimens were examined from 18 patients with protoporphyria, 6 of whom died in hepatic failure with cirrhosis. All of the fatal cases have been previously reported [15,17,18,19,22]. Biopsy specimens from 5 of the 12 nonfatal cases were supplied by Dr. Derek Cripps of the University of Wisconsin and Dr. Peter Scheuer of the Royal Free Hospital, London [25]. In each case, the diagnosis of protoporphyria was made on the basis of life-long photosensitivity and elevated red cell protoporphyrin levels. Several of the patients had other family members with the disease.

Cirrhosis was present in each of the fatal cases. In addition, there was active hepatocellular necrosis, portal inflammation, and cholestasis. Characteristically, there were massive deposits of dark brown pigment in Kupffer cells, portal histiocytes, bile canaliculi, and parenchymal cytoplasm (Fig. 2). When examined by polarization microscopy, the pigment deposits showed striking birefringence (Fig. 2). Large deposits were bright red with a central dark Maltese cross, whereas the smaller deposits appeared as clusters of brilliantly illuminated granules. Ultrastructurally, the deposits were composed of crystalline material which lay free in the cytoplasm of the hepatocytes, but in Kupffer cells were exclusively in lysosomes (Fig. 2).

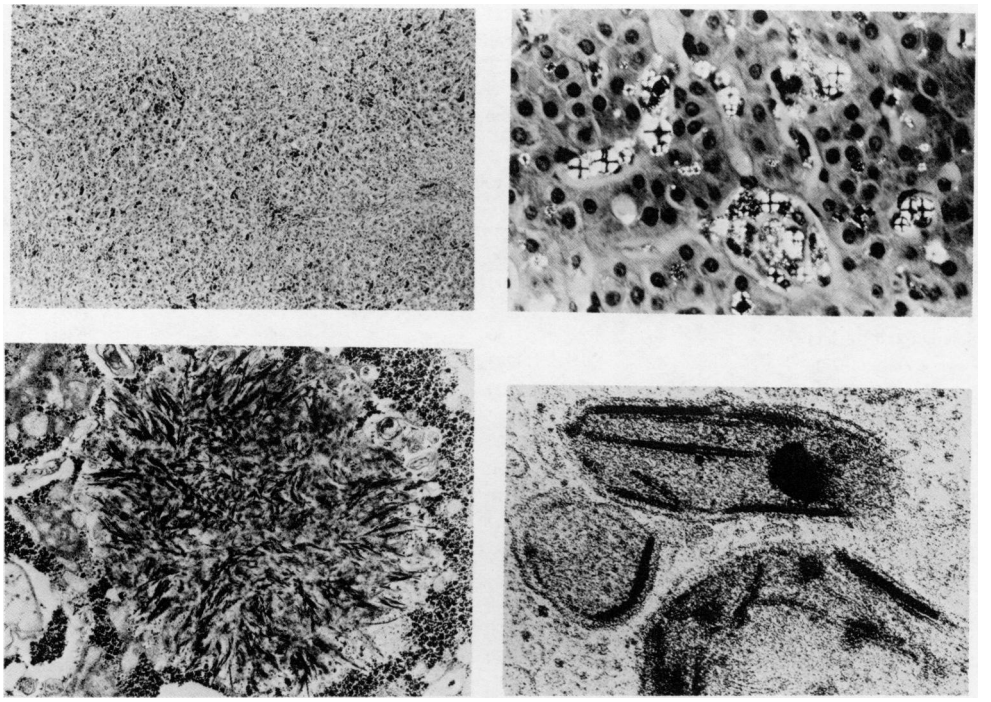

FIG. 2. Hepatic protoporphyrin deposition in a case of protoporphyria with cirrhosis and fatal hepatic failure. *Top left:* Diffuse pigment deposits in parenchyma. Hematoxylin and eosin stain. *Top right:* Polarization microscopy of pigment deposits showing birefringence with centrally located dark Maltese crosses. Hematoxylin and eosin stain. *Bottom left:* Part of one hepatocyte contains a star-burst deposit made up of amorphous material and electron-dense crystals arranged radially. Lead hydroxide stain. *Bottom right:* Several crystals are shown within the lysosomes of a Kupffer cell. Lead hydroxide stain. Reprinted with the permission of The American Journal of Medicine (Bloomer et al, Am J Med 58:869–882, 1975).

Needle liver biopsy specimens were also examined from 12 nonfatal cases (Table 2). These cases were not randomly selected and do not indicate the frequency with which hepatic lesions will be found in patients with protoporphyria at large. Birefringent pigment deposits were present in 7 of the cases. The deposits were found in portal triads, and there was portal inflammation with a variable degree of fibrosis. In 4 of the 7 cases, there was also parenchymal pigment deposition accompanied by hepatocellular necrosis. None of the cases without birefringent pigment deposits had hepatocellular necrosis. However, 2 showed portal inflammation and fibrosis.

There was no evidence of iron overload in any of the 18 patients, as evaluated by iron stains of the liver biopsy specimens.

TABLE 2
Lesions in Needle Liver Biopsy Specimens of Patients with Nonfatal Protoporphyria

	Birefringent pigment deposits present (7 cases)	Birefringent pigment deposits absent (5 cases)
Portal inflammation ± fibrosis	7	2
Hepatocellular necrosis	4	0
Cirrhosis	0	0

TABLE 3
Hepatic Heme Synthetase (HSyn) and δ-aminolevulinic Acid Synthetase (ALAS) Activities in Protoporphyria

Case	Liver Histology	HSyn (pmoles heme/mg protein/hr)	ALAS (pmoles ALA/mg protein/hr)
1	Normal	508	—
2	Normal	906	—
3	Portal inflammation	440	—
4	Portal inflammation	297	853
5	Cirrhosis	91	—
	Mean ± SEM	448 ± 135	
Controls (mean ± SEM)		2078 ± 298[a]	476 ± 70[b]

[a]10 patients [b]6 patients

Hepatic Heme Synthetase and ALA Synthetase Activities in Protoporphyria

Heme synthetase activity was measured by a radiochemical assay [5] in liver biopsy specimens from 5 patients with protoporphyria (Table 3). The mean activity was significantly reduced compared to that in 10 control biopsy specimens with similar degrees of hepatocellular damage ($p < 0.001$).

The activity of ALA synthetase, the first and rate-limiting enzyme of the heme biosynthetic pathway, was measured by a radiochemical assay [26,27] in the liver biopsy specimen of one patient with protoporphyria. It was mildly increased compared to that in 6 control specimens (Table 3).

Therapy of Hepatic Disease in Protoporphyria

Serial observations in one patient have provided the opportunity to evaluate whether hepatic disease in protoporphyria can be reversed. The result of the initial evaluation of this patient, as well as that of her 33-year-old sister who died with protoporphyria and liver failure, has previously been published [17]. Her case history is as follows:

This 26-year-old housewife had severe photosensitivity since childhood. She never had signs or symptoms of hepatobiliary disease. She had one uncomplicated pregnancy, delivering a healthy female child. The patient did not drink alcoholic beverages. She had taken birth control pills (Ovral) but did not use other medicines. During that period her photosensitivity did not worsen. The birth control pills were stopped one week before her admission to Yale–New Haven Hospital.

Physical examination was normal, except for mild scarring over the lips and thickening of the skin over the dorsal aspects of her hands and forearms. There were no stigmata of liver disease. The serum glutamic oxaloacetic transaminase (SGOT) level was 69 units (normal less than 35), and bromosulfalein (BSP) retention at 45 min was 12% (normal less than 5%). The serum bilirubin was 0.86 mg/100 ml, alkaline phosphatase 50 IU/L, cholesterol 195 mg/100 ml, and albumin/globulin 4.4/3.1 g/100 ml (all normal). Hematological values were normal. Serum iron was 92 μg/100 ml, with a total iron binding capacity of 496 μg/100 ml. The serum did not contain hepatitis B antigen or antibody, smooth muscle antibody, or mitochondrial antibody. Oral cholecystogram was normal. Liver scan with technetium sulfur colloid showed hepato-splenomegaly without focal defects. The protoporphyrin concentrations in her red cells and plasma were 1426 ± 212 μg/100 ml red cells and 123 ± 46 μg/100 ml plasma (mean ± SEM of 5 fasting blood samples) (normal less than 50

μg/100 ml red cells and 10 μg/ml plasma). Fecal excretion of protoporphyrin was 38 mg/24 hr in a 72 hr stool specimen (normal less than 4 mg/24 hr).

Needle liver biopsy revealed normal lobular architecture, but all portal triads were enlarged with occasional fibrous bridging. The triads showed erosion of the limiting plates and contained a variable number of lymphocytes, pigmented macrophages, and globules of brown pigment. Central zones showed focal loss of hepatocytes which were replaced by collections of pigmented macrophages and lymphocytes. Rare canaliculi contained pigment deposits as well. When examined by polarization microscopy, the pigment deposits showed birefringence. None of the hepatocytes examined by electron microscopy contained crystalline deposits. However, the lysosomes were prominent. Numerous crystalline deposits were observed in lysosomes within Kupffer cells. Iron stain of the liver revealed normal iron stores.

The patient was started on cholestyramine (12 g/day of Questran in divided doses) and vitamin E (100 units of Aquasol E daily). The first period of therapy extended for 11 months (Fig. 3). Her plasma protoporphyrin concentraion fell rapidly after starting therapy. The mean concentration during the period of therapy was 16 μg/100 ml, only 13% of the pre-therapy value, and the majority of levels were normal. The red cell concentration diminished at a slower rate than the plasma level. By the end of therapy the level was only 30% of the pre-therapy value. Fecal excretion of protoporphyrin similarly declined.

The serum transminase and BSP retention returned to normal during this period. There was no change in the patient's serum iron level or hematological values. Her photosensitivity was dramatically decreased.

Needle liver biopsy at the end of this period showed that several portal triads still had scarring and increased numbers of ductules and pigmented histiocytes. Some central areas had zones of collapse. However, there was a marked decrease in the amount of necrosis, inflammation, and fibrosis compared to the pre-therapy biopsy. Moreover, the amount of birefringent pigment demonstrable by polarization micros-

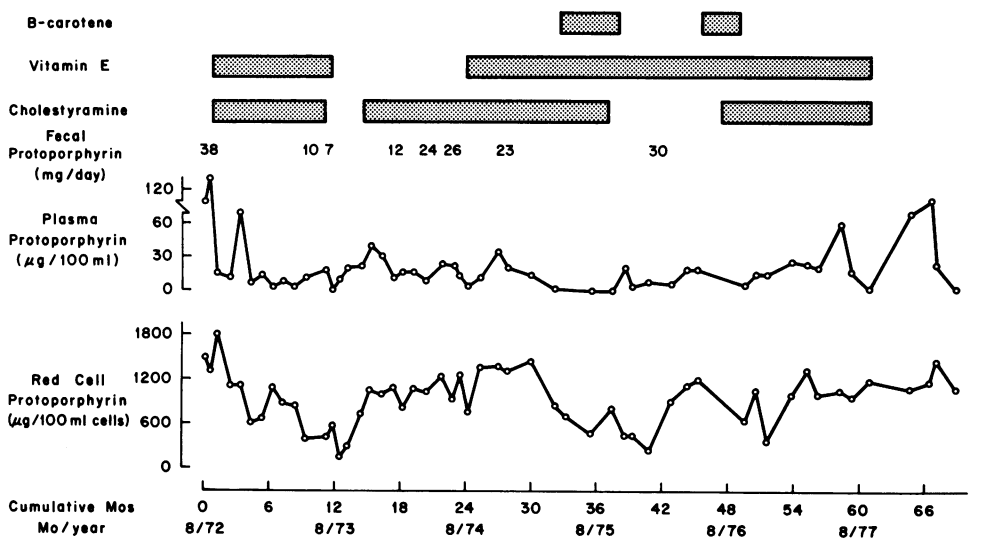

FIG. 3. Course in a patient with protoporphyria who was treated with cholestryamine (12 g Questran daily) and vitamin E (100 units Aquasol E daily) because of hepatic damage. In addition, Beta-carotene was given on two occasions to treat photosensitivity. See text and Fig. 4 for effects of therapy on liver function tests and liver histology.

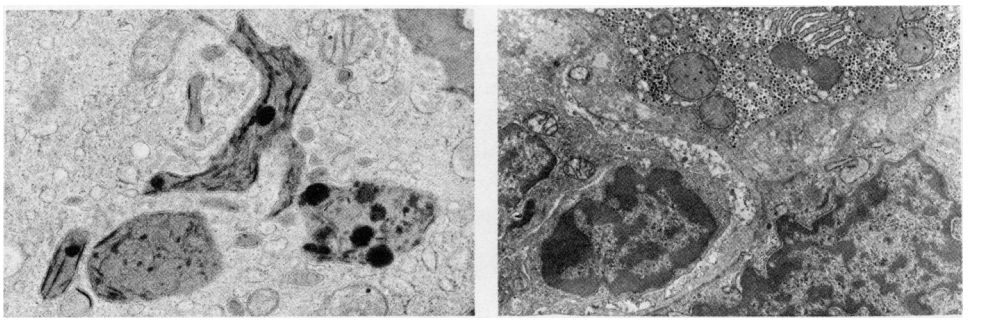

FIG. 4. Pre-therapy liver biopsy specimen of patient in Fig. 4 is compared with that after the first year of therapy. *Left:* Part of one Kupffer cell in the pre-therapy biopsy specimen shows lysosomal granules stuffed with amorphous material, electron dense droplets, and numerous elongated crystals. Lead hydroxide stain. *Right:* Part of several Kupffer cells is shown in the post-therapy liver biopsy specimen. Crystalline material is absent. Lead hydroxide stain.

copy was considerably less. The hepatocytes appeared ultrastructurally normal. The prominent hepatocellular lysosomes seen previously were now reduced to normal size. The Kupffer cells were also normal, and crystalline deposits were not found in their lysosomes (Fig. 4).

When the patient was withdrawn from therapy for 3 months, there was a steady increase in the red cell protoporphyrin level, with a lesser increase in the plasma level (Fig. 3). She was subsequently restarted on therapy. Liver function tests have continued to be normal for the remainder of her course. Liver biopsy has not been repeated.

DISCUSSION

Protoporphyrin is formed by sequential decarboxylation of the porphyrin side chains (Fig. 1). Uroporphyrin, which has 8 carboxyl groups, is converted to protoporphyrin, which has 2 carboxyl groups, causing the molecule to be less water soluble and changings its route of excretion. Uroporphyrin is excreted predominantly in urine, coproporphyrin in both urine and bile, and protoporphyrin solely in bile [1]. The mechanism by which hepatic excretion of protoporphyrin occurs has not been defined. Part of the excretory pathway may be the same as that for other organic anions such as bilirubin. Protoporphyrin is not changed to a more polar derivative by hepatic metabolism, however.

In protoporphyria, protoporphyrin accumulates in excessive amounts because of the reduced activity of heme synthetase. Erythropoietic tissue appears to contribute the major fraction. Free protoporphyrin rapidly leaves newly formed red cells when they enter the circulation and may account for the protoporphyrin which is excreted in stool [7]. Since heme synthetase activity is deficient in hepatic tissue of patients with protoporphyria (Table 3), the liver may also contribute to protoporphyrin accumulation. This possibility is supported by studies which have utilized labeled precursors of protoporphyrin [8,9], although the interpretation of these studies is controversial [28].

Regardless of the sites of protoporphyrin accumulation, the liver must excrete the excess amount. Liver damage may occur when crystalline protoporphyrin deposits form in hepatobiliary structures. Cholelithiasis has been reported in patients with protoporphyria [25,29], and the gallstones have contained high levels of protoporphyrin. Protoporphyrin also precipitates in intrahepatic bile ducts and bile canaliculi.

Either extrahepatic or intrahepatic biliary precipitation of the compound could cause portal inflammation and fibrosis by biliary obstruction or by a toxic effect of the crystalline material on bile duct epithelium. Crystalline deposits of protoporphyrin also form within hepatocytes (Fig. 2), a process which may cause cell death. As liver damage is initiated and cholestasis develops, protoporphyrin accumulation in the liver may increase rapidly. The common history of patients with protoporphyria who have died in liver failure is that death occurred within a few months after the onset of jaundice.

Since significant liver disease occurs in a small percentage of patients with protoporphyria, it is important to identify susceptible individuals. Liver function tests may be minimally abnormal in patients in whom the fatal cycle is imminent [10–22]. Fecal protoporphyrin excretion has not been correlated with the potential to develop liver damage, but red cell protoporphyrin levels have been very high in patients who have died with cirrhosis and liver failure (Table 1). This may reflect the fact that protoporphyrin is in a dynamic flux between red cell, plasma, and liver compartments. In the animal in which experimental porphyria has been produced, red cell protoporphyrin levels increase linearly after the hepatic concentration reaches 100 μg/g wet weight [30], a level which is much lower than that found in patients with fatal protoporphyria (Table 1). For these reasons, liver biopsy is indicated to evaluate patients with protoporphyria who have abnormal liver function tests, even of minimal degree, and markedly elevated red cell and plasma protoporphyrin levels.

Therapy of hepatic disease in protoporphyria should be directed at depleting hepatic porphyrin stores and diminishing the amount of protoporphyrin which the liver has to excrete. One approach is to decrease the rate of protoporphyrin production. Factors which may adversely affect heme synthetase activity, such as iron deficiency [31], should be corrected. Fasting should be avoided [14], since measurements of fecal protoporphyrin excretion suggest that protoporphyrin production increases during caloric restriction [32]. Infusion of hematin, which diminishes levels of porphyrin precursors in urine and blood of patients with acute intermittent porphyria by negative feedback repression of ALA synthetase [33], may decrease protoporphyrin overproduction in protoporphyria [34]. Vitamin E administration has also been proposed as a means by which to diminish porphyrin production in porphyric disorders [35], although studies of its effect have produced inconsistent results [36,37].

A second line of therapy is to interrupt the enterohepatic circulation of protoporphyrin [38] by administering cholestyramine, a nonabsorbable compound which binds anionic compounds [39]. Kniffen [40] and Lischner [41] reported that cholestyramine therapy reversed hepatic disease in patients with protoporphyria.

Therapy with cholestyramine and aqueous vitamin E was associated with significant improvement in our patient. Protoporphyrin levels decreased (Fig. 3), liver function tests returned to normal, and the hepatic lesion resolved (Fig. 4). However, caution must be used in attributing the improvement entirely to the administration of these compounds. It is conceivable that she had spontaneous improvement, although the pattern of her response to therapy makes this unlikely. Second, she had been taking birth control pills for two years before her initial evaluation. Birth control pills do not produce the hepatic lesion which was present in her biopsy specimen but they might impair the hepatic excretion of protoporphyrin, thereby contributing to the hepatic damage indirectly.

The fact that protoporphyrin levels in red cells, plasma, and liver of our patient all diminished during therapy indicates there was an overall decrease in tissue porphyrin stores. It is not possible to evaluate the relative contributions of enhanced protoporphyrin excretion and decreased protoporphyrin production to this change. However, the observations do indicate that excretion of protoporphyrin was greater than the rate of production during this period.

This patient's course demonstrates that hepatic damage in protoporphyria may be reversed if detected at an early stage. Improvement was associated with a decrease in hepatic pigment deposits, lending further support to the concept that liver disease in protoporphyria is caused by the deposition of protoporphyrin in hepatobiliary structures.

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